Noteworthy anatomical and physiological researches.

The fixation of free nitrogen by plants.

A review of the question of the assimilation of free nitrogen must of necessity be somewhat disconnected as different investigators approach the subject from such different standpoints, some dealing entirely with the economic phase while others treat its biological aspect.

The present synopsis deals mainly with the literature of the year 1893, including only those papers of the previous year that throw light upon concluding investigations.

Research on this question has not been as active during the past year as in preceding years, and in a number of cases papers are only concluding pieces of work undertaken earlier. The general trend of the whole subject, broadly considered, has been much more in the line of general physiological experiment than in the morphological study of the agent of nitrogen fixation.

Assimilation by non-leguminous plant organisms.

Concerning the question as to what organisms are able to utilize uncombined nitrogen, several papers have appeared.

Frank's contribution to the subject in showing that some of the algæ possess this ability seems now to be settled beyond dispute. Schlœsing and Laurent [1] experimented upon this question, using both the direct and indirect methods of nitrogen determination and found that not only were the green algæ able to fix gaseous nitrogen but that some of the mosses possessed this peculiarity in a marked degree. Koch and Kossowitsch [2] have repeated this work with green and blue green algæ, using purely inorganic solutions and have arrived at the same conclusion. While the number of experiments upon this point seem to show conclusively that the lower green forms of vegetable life possess this power, yet it would seem desirable if experiments were also carried out with pure cultures of various forms and thus thoroughly exclude all possible chance for misinterpretation of results. [3]

[1] Ann. Inst. Past. **6**: 110. 1892.–Comptes rendus Acad. **115**: 732. 1892.
[2] Bot. Ztg. **51**: 342. 1893.
[3] Some of Schlœsing and Laurent's experiments were carried out on approximately pure species but they were not grown in sterilized culture media.

The importance of algal assimilation, for so long a time overlooked, is by no means inconsiderable, for it doubtless will enable one to harmonize many results that heretofore seemed inexplicable. Particularly is this true with experiments carried on in natural soils with non-leguminous phanerogams, where the nitrogen claimed to be assimilated is always relatively small.[4]

In regard to chlorophylless organisms, Berthelot[5] has recently studied several soil bacteria in pure culture, *Aspergillus niger*, *Altenaria tenuis*, and a Gymnoascus, using for a culture medium, humic acid and kaolin. With these forms he was able to detect a marked increase in the nitrogen content. The bateria of lupine tubercles grown in humic acid and Cohn's solution increased the amount of fixed nitrogen by fifty per cent. He also noted that when the amount of combined nitrogen becomes large, the organisms utilize this rather than continue to fix the elemental gas.

Winogradsky[6] has issued a preliminary paper upon the ability of bacteria to function as nitrogen collectors. He worked under bacteriological conditions, using for a culture medium a non-nitrogenous but fermentable solution (pure dextrin and specially prepared mineral salts). With this medium, he isolated one well characterized bacillus able to form gas and produce butyric acid in quantities. It would grow neither on gelatin nor on gelatinized silica to which sugar had been added. In general, it bore a strong resemblance to Fitz's *B. butylicus*.

While the evidence at hand as to the ability of lower organisms to utilize atmospheric nitrogen seems to be fairly complete, it is not so definitely settled whether the same is true for higher plants, excluding of course the legumes. Frank has persistently maintained the view that the ability of fixing nitrogen was a function of protoplasm and was resident in the higher plants as well as the simpler. Especially is this marked, he claims, in thrifty, vigorous plants in the growth subsequent to the seedling stage. He has found, repeatedly, a marked increase in the nitrogen content of soil and crop where non-leguminous plants such as rape, oats and

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[4] Note in this connection the results of Schlœsing and Laurent, Ann. Past. **6**: 110, in which a fixation of N was observed with oats, cress and mustard, where algal vegetation flourished, but where this was excluded no gain could be detected.

[5] Comptes rendus Acad. **116**: 842. 1893.

[6] Comptes rendus Acad. **116**: 1385. 1893.

mustard were used. In his last paper,[7] he brings together the results of several experiments made during the last few years, in which is shown a gain in N, both in crop and soil, over what was in the seeds and soil at the beginning. Frank's methods are not given in sufficient detail to enable one to judge of his results critically; in fact this charge has been made repeatedly against many of his observations.[8] He uses mainly the indirect method of nitrogen determination, planting the seeds in a soil containing a known quantity of fixed nitrogen and then determines by analysis the content of the soil and crop. If the sum at the end exceeds the total amount available at the beginning, he reasons that the plant has assimilated gaseous nitrogen. A control pot with unplanted soil is usually analyzed to see if there is any change in the fixed nitrogen of bare soil. As his experiments are usually carried out on unsterilized soil and his unplanted check soils often show a gain in fixed nitrogen, there is hardly any doubt that the N-increase in his experiments with non-leguminous plants is in part due to fixation by lower organisms, algæ, fungi, or bacteria that are common to the soil.

Kreusler[9] points out a serious objection to his methods of analysis as not sufficiently accurate to discriminate in the case of non-leguminous plants where such small increments are to be noted.

In the résumé above referred to two experiments with non-leguminous phanerogams are given which were made in absolutely N-free land, the results of which are as follows:

Sinapis alba (4 plants): grams of N in seed, 0.0012; in crop, 0.0043.
Solanum tuberosum (4 pieces): grams of N in seed, 0.022; in crop, 0.2186.

He also describes a still more recent experiment, made with *Sinapis alba.* In this case he used large bell jars and although the plants did not develop normally (they were unable to unfold their flower buds in this closed space), he found a certain amount of nitrogen fixed.

The N content at beginning was as follows:

Three seeds, 0.0009^{gm}; soil, 0.162%;

at close of experiment,

Crop, 0.0507^{gm}; soil of pot, 0.215%; soil of control, 0.195%.

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[7] Bot. Ztg. **51**: 150. 1893.
[8] Journ. f. Landw. **41**: 144. 1893.
[9] Bied. Cent. **21**: 257.

Frank claims that the results reported by Liebscher[10] with mustard are in general confirmatory of his experiments. This latter investigator worked under field conditions and claims that upon rich soil white mustard can collect twice as much nitrogen as thrifty peas, beans or clover. Liebscher's paper is very full and explicit as to methods and details but his experiments were conducted under such conditions that the different factors were not controlled, hence the value of the conclusions is much lessened. He concludes that the ability of collecting nitrogen is present with certain non-leguminous plants (mustard and oats) but only when they are growing under optimum conditions. In rich unsterilized soil, peas do not increase in thriftiness even if they are fed with combined nitrogen, while the non-leguminous plants are much benefited by such a treatment.

Liebscher thinks that errors of analysis will hardly explain the quantity of nitrogen apparently collected by the oats and mustard but as he admits, no control was exercised over the rainfall or the water used for watering, neither was the influence of algæ or soil organisms taken into consideration.

It would seem that while there may be an increase in the nitrogen under field conditions that may possibly possess some economic value, yet from the standpoint of physiology, these experiments are not sufficiently conclusive to prove that the higher plants themselves were able to fix the nitrogen.

Lotsy[11] has recently studied this question relative to the mustard assimilation in a careful way, employing both sand and water cultures in sterile and unsterilized condition, and from his work concludes that neither *S. alba* nor *S. nigra* are able to live without combined nitrogen. In this connection it is only necessary to refer to the exceedingly careful researches made previous to this by Schlœsing (fils) and Laurent[12] in which they showed by a comparative set of experiments, by both direct and indirect methods of analysis, that white mustard, oats, cress, and spergula were unable to assimilate free nitrogen.

In 1890 Petermann[13] affirmed that barley was as efficient a nitrogen collector as beans. Since then he has published a second paper[14] giving full details of his experiments. His

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[10] Journ. f. Landw. **41**: 180. 1893.
[11] Bull. O. E. S. Dept. of Agriculture *18*.
[12] Ann. Inst. Past. **6**: 114. 1892.
[13] Mém. Acad. roy. de Belg. **44**: 1889.
[14] Mém. Acad. roy. de Belg. **47**: 1892.-Abs. in Chem. Cent. **2**: 988. 1893.

plants were grown in natural soil, under normal atmospheric conditions, and also in air freed from combined nitrogen. His results showed a marked gain with barley in normal air, and somewhat less increase in air freed from fixed nitrogen. The N-content of seed, water added, drainage water, and crop were carefully determined but as he himself says, the factor of unsterilized soil does not exclude the possibility that lower organisms may have functioned in the capacity of nitrogen collectors. He has since repeated his experiments,[15] using both natural and sterilized soils, and arrives at a different conclusion. In unsterilized unplanted controls, having, however, evident algal growth, a slight gain was noted. In sterilized unplanted soil and soil sown to barley a slight reduction was found. This corroborates Schlœsing's results and shows that the increase sometimes ascribed to arable land is really due to its living organisms. Unfortunately, the experiment in unsterilized soil planted to barley was lost, but the fact that the sterilized soil planted with barley lost a part of its N shows that the supposed gain in the previous series was really due to soil organisms of a lower type.

In Frank's last paper,[16] already referred to, he presents his views in a compact and well digested form, citing experiments of his own, some of which are detailed for the first time, and critically reviewing the work of other investigators. He regards the experiments carried on in closed glass spaces as unnatural inasmuch as the conditions are so abnormal that the plant is unable to fruit. As he claims that the nitrogen assimilation of non-leguminous plants can only take place when the plant is thrifty and vigorous, this objection seems well founded. As conditions more nearly approaching those of the open air necessarily embrace influences that must be considered, it would seem that the only way to settle this question is to carry out simultaneous experiments under various conditions by both direct and indirect methods and then correlate the results.

Frank summarizes his results as follows:

1. The legumes can assimilate free N without the intervention of the symbiotic organism.

The strongest case he cites to prove this is the experiment made with four plants of *Robinia pseudacacia* in N-free steril-

[15] Bull. Acad. roy. de Belg. **25**: 267–276. 1893.
[16] Bot. Ztg. **51**: 139. 1893.

ized sand, in which an increase in nitrogen from 0.0024^{gm} to 0.0538^{gm} is noted. This experiment he regards as fatal to the theory of Hellriegel, inasmuch as this legume without tubercles on its roots can materially increase its nitrogen supply.

2. The symbiotic microbe isolated from a leguminous plant thrives luxuriantly on organic N but barely lives when it derives its N from the air.

In this view he is opposed more or less strongly by Prazmowski,[17] Laurent,[18] Beyerinck,[19] and Bertholot,[20] all of whom maintain that pure cultures of the tubercle organisms take up quantities of uncombined nitrogen.

3. The quantity of combined N in root tubercles does not suffice to account for the N in remaining plant organs.

He takes the analyses of five plants of *Lupinus luteus* and determines the N-content of the tubercles, the aerial organs and the roots proper and shows that at no time during the development of the plant do the tubercles contain more than a fraction of the nitrogen that is present in the plant. Unless the tubercles yield up a continuous supply of N, which has never been claimed as taking place, it is hardly possible to account for the N supply of the plant unless the plant itself takes part in the assimilatory process. As the samples selected were taken from an open field, the conditions are such that the conclusion is hardly warranted that the plant itself assimilated a large part of the nitrogen. Frank's own experiment with this same plant in sterilized soil (sand) only showed with six plants an increase from 0.042^{gm} in seed to 0.3475^{gm} N in crop, so that the factor of soil and its organisms seems to be more important than anything else even in his own experiments.

4. The non-leguminous vegetable organisms can assimilate free nitrogen.

To show how wide spread is this function, he classifies examples under the following heads, including:

(*a*) fungi, quoting as an example a ten months' culture of *Penicillium cladosporioides* in a nitrogen-free sugar solution as fixing 0.0035^{gm} of N;

(*b*) algæ and mosses;

(*c*) phanerogams;

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[17] Landw. Versuchst, **38**: 5. 1891.
[18] Ann. Inst. Past. **5**: 135. 1891.
[19] Bot. Cent. **52**: 137. 1892.
[20] Comptes rendus Acad. **116**: 842. 1893.

citing a résumé of the experiments he has made with different plants. He also quotes confirmatory evidence from Liebscher and Petermann that may now be disregarded or at least considered of very doubtful value.

5. How far is combined N (nitrate), if used as a manure, utilized by the plant and what becomes of it in the soil?

Frank holds that this subsidiary question should also be considered in a discussion of the nitrogen question. Most agriculturists affirm that if plants are fed with increasing amounts of nitrates a corresponding increase in N will be found in the crop. Frank planted mustard in N-free soil to which definite amounts of $Ca(NO_3)_2$ were added.

The seed contained .0003^{gm} and the soil .061^{gm} in the form of the salt, while the crop showed .051^{gm} N and no trace was found in the soil. An unplanted check soil containing .061^{gm} at the same time contained only .0046^{gm} N, thus showing that there is a large loss that is of no use to the plant.

Repeating a part of the experiment with unplanted soil pots to which a definite amount of the nitrate had been added, a large portion of the nitrogen was found to have disappeared. This disappearance he thinks is due to activity of micro-organisms of the denitrifying type as shown by Breal,[21] Schlœsing and others, but it shows that the increased amounts of N furnished in a manure may not reappear in the crop. They serve to make the plant more thrifty in the beginning and thus increase its ability to utilize free nitrogen. For this reason it is necessary to furnish combined nitrogen to non-leguminous plants during the germinating period while the legumes on the other hand can forego fixed nitrogen from the first, owing partly to their highly albuminous seeds and partly to the symbiotic relations that they maintain with the tubercle organism by means of which the assimilatory activity of the plant is largely increased.

The actual fixation of nitrogen by legume tubercles.

Concerning the ability of legumes to acquire free nitrogen there is no diversity of opinion, but just how these plants fix this gaseous element is not so definitely known. The generally accepted idea that the process bears an intimate relation to the presence of root nodules is no doubt correct in the main, but whether the nitrogen is fixed by the nodule organ-

[21] Comptes rendus Acad. **114**: 681. 1892.

ism or the plant itself or is a partnership act is by no means settled.

The most important contributions to this phase of the question that have appeared in the past year are the joint papers of Nobbe and Hiltner.[22] In their several papers, covering experiments since 1890, they show concordant results. They hold that the assimilation of nitrogen by legumes bears a direct relation to the formation of bacteroids. In numerous cases they found that plants (peas) growing in good soil and well supplied with nodules were unable to make much gain. When inoculated with pure cultures of *B. radicicola*, some plants would gain largely in nitrogen while others would apparently suffer from nitrogen hunger. When the tubercles on these plants were carefully examined they noted that the nodule-producing organisms were unchanged in those plants that hungered for nitrogen, while in the thriftier ones, the bacteria were changed into bacteroids. They conclude that (1) tubercles in which bacteroid formation does not occur are injurious instead of beneficial to the host plant, (the unchanged bacteria are then merely parasites); (2) the unchanged bacteria present in tubercles seem to have no relation to the nitrogen fixation by legumes; (3) the more vigorous the bacteria the less tendency there is toward bacteroid formation; (4) the assimilation of N begins with the formation of bacteroids.

In some experiments with Robinia they obtained striking results. The plants gained more nitrogen in the end when cultivated in nitrogen-free soil than in soil containing nitrogen. This was because there was a more complete conversion of the bacteria into bacteroids in non-nitrogenous soil than where nitrates were present. Manuring with nitrates causes a more rapid development of the plants at first, and with this a more rapid growth of smaller nodules, but these were of less benefit than the larger nodules noted in nitrogen-free soil, the bacteria of which were entirely changed into bacteroids.

The formation of bacteroids in the light of this view will have then an increased interest.

Nobbe and Hiltner claim that the bacteroids are formed by repeated division of the tubercle germ without the separation into isolated individuals. This continued division usually

[22] Sächs. landw. Zts. *16:* 165. 1893. Landw. Vers. Stat. **42**: 459. 1893.

takes place transversely, and this produces an elongated growth although lateral protuberances often arise making a branched and irregular appearance. They liken the swollen branched bacteroids to a gill respiration, the nitrogen being absorbed by the water and this coming to the absorbing surfaces in a dissolved condition. The fact that nodules are less active in their assimilatory capacity in water cultures than in soil is commented upon and the inference drawn that the slower exchange of gases in the water than in capillary soil is the cause of this lessened activity.

Variety of species of nodule-producing organism.

Regarding the question as to whether there is a variety of species of the nodule forming organisms, Nobbe and Hiltner give some additional experiments in infecting different legumes with bacteria normally found in other species.[23] In nitrogen-free soils, certain plants like *Lupinus luteus*, *L. augustifolius*, *Acacia Lophantha* and *A. Julibrissin* produced tubercles when inoculated with bacteria of pea and bean tubercles. In soil containing nitrogen no infection could be noted, indicating that there must be a nitrogen hunger in the plant before the tubercle bacteria of one species of legume can penetrate the root system of another species.

Atkinson[24] records in his paper the failure to produce tubercles on *Dolichos sinensis* when inoculated with pure cultures isolated from *Vicia sativa* while this organism introduced into its normal host developed abundant tubercles.

The multiplicity of forms that have been noted among the bacteroids of different legume species has led to the view that there are specific forms for different species of legumes. This view receives support from a morphological basis but the uncertainty of a classification based upon a possible involution or abnormal structure is obvious.

Schneider[25] classified the tubercle organisms under the generic title of Rhizobium, adopting Frank's generic name. He based this classification at first on purely morphological characters as they appeared in the living tubercle, but he has since cultivated several forms artificially and has added cultural characteristics to his morphological data.

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[23] For earlier data on this question see Landw. Vers. Stat. **39**: 227–359. 1893.
[24] Bot. Gaz. **18**: 157. 1893.
[25] Ber. d. d. bot. Ges. **12**: 11. 1894.

Atkinson has suggested that the influence of the macro-symbiont upon the tubercle organism may have much to do with the variability of form as seen in the different types of bacteroids.

Bearing upon this question of variety of species are the very interesting observations of Bolley[26] on the natural distribution of tubercles on the roots of indigenous and introduced legumes of the western plains. The native flora of the region is distinctively leguminous and he gives a list of native forms that he finds well provided with tubercles under natural conditions of environment. Many of the introduced legumes, especially *Trifolium pratense* often fail to establish themselves in the Dakotas for some reason. On stray plants, self-seeded and alone, he finds few, if any, tubercles, even though they may be growing in the midst of the native leguminous forms, but when preceded by *T. repens* this form develops nodules on its roots and is apparently thrifty. Several other introduced legumes fail to produce tubercles when planted on the virgin soil.

The inability of these species to produce nodular outgrowths would seem to favor the theory that their special organism was lacking and therefore would indicate that there is a variety of species. These observations have, however, only a circumstantial value in lieu of actual infection experiments.—H. L. RUSSELL.

The influence of traction upon the growth of plants.

Hegler in a recent paper[1] points out some of the work done on this subject by other investigators: (1). Baranetzky concluded that the duration and intensity of growth were in no wise affected by traction. (2). Max Scholtz thought that the effect of traction was twofold: (*a*) a retardation which he considered a pathological effect; (*b*) an acceleration in which he saw the real mechanical effect of the traction. The author advances some important objections to these conclusions and then gives an account of his own investigations.

Method.—Two plants were used, one with and one without a weight. Measurements were taken by means of distance marks, microscope and micrometer, or by the Bara-

[26] Ag. Science **7**: 58. 1893.

[1] HEGLER, ROBERT. Ueber den Einfluss des mechanischen Zugs auf das Wachsthum der Pflanze. Beiträge zur Biologie der Pflanzen, **6**: 281. 1894.